Jurnal Teknologi

Full paper

Effect of Particle Size on the Oil Yield and Catechin Compound Using Accelerated Solvent Extraction

Mohd Azizi Che Yunus[a*], Manzurudin Hasan[a], Norasikin Othman[a], Siti Hamidah Mohd-Setapar[a], Liza Md.-Salleh[a], Muhammad Abbas Ahmad-Zaini[a], Zuhaili Idham[a], Salman Zhari[a]

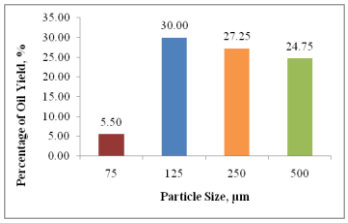
[a]*Centre of Lipid Engineering Applied Research, CLEAR, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

*Corresponding author: azizi@cheme.utm.my

Article history

Received :5 January 2012
Received in revised form : 6 August 2012
Accepted :5 December 2012

Graphical abstract



Abstract

The purpose of this work is to investigate the effects of particle size on the extraction of catechin compound from *Areca catechu L.* seeds by using Accelerated Solvent Extraction (ASE). The particle sizes of *Areca catechu L.* seeds are varied from 75 µm until 500 µm. The extraction is conducted at fixed parameters which are temperature (140°C), pressure (1500 psi), extraction time (10 minutes), flush volume (60%) and the static cycle is done for 1 extraction cycle respectively. Higher percentage oil yield of 300 mg oil/gram of sample (30.00% oil yield) is found at 125 µm. However, the amount of catechin in oil yields is only 0.0375 mg of catechin/gram of sample. The best of particle size within the experimental range has been identified at 500 µm which gives a high content of catechin with 0.0515 mg Catechin/gram of sample from 247.5 mg oil/gram of sample (24.75% oil yield).

Keywords: Particle size; catechin; LC-MS-TOF; accelerated solvent extraction

Abstrak

Kajian ini bertujuan untuk mengkaji kesan saiz zarah ke atas pengekstrakan sebatian catechin daripada biji *Areca catechu L.* dengan menggunakan Pengekstrakan Pelarut Terpecut (PPT). Saiz zarah biji *Areca catechu* dipelbagaikan dari 75 µm sehingga 500 µm. Pengekstrakan telah dijalankan pada parameter tetap iaitu suhu (140°C), tekanan (1500 psi), masa (10 minit), isipadu semburan (60%) dan satu kitaran pengekstrakan, masing-masing. Hasil minyak peratusan yang lebih tinggi adalah 300 mg minyak / gram sampel (30.00% pati minyak) ditemui pada 125 µm. Walaubagaimanapun, kandungan catechin dalam pati minyak hanya 0.0375 mg catechin / gram sampel. Saiz zarah yang terbaik dalam julat uji kaji ini telah dikenal pasti pada 500 µm yang memberikan kandungan catechin yang tinggi iaitu 0.0515 mg catechin / gram sampel dari 247.5 mg minyak / gram sampel (24.75% pati minyak).

Kata kunci: Saiz zarah; catechin; LC-MS-TOF; pengekstrakan pelarut terpecut

1.0 INTRODUCTION

In Malaysia, *Areca catechu Linn* is known as "Pinang" and it is a familiar masticate and appetizer eaten by local people. Most of the people like to masticate the betel nut and the history shows that this practice was first recognized among Indians in the past 2000 years (Azeez *et al.*, 2007). *Areca catechu L.* is part of the Arecaceae family. There are over 200 classes and about 2600 species in the Arecaceae family (Lee *et al.*, 1999). *Areca catechu L.* seeds contain a valuable chemical constituent which is catechin. Catechin is considered as a powerful dietary antioxidant. The major chemical compounds that contribute as antioxidant for the benefits of human health are Polyphenols, which consist of catechin and phenolic acids (Ho *et al.*, 1992). The chemical constituents in *Areca catechu L.* are not only used for medicinal purpose, they can also be used as natural dye as well. Tannins contain a catechin monomer, known as dye pigment which gives a reddish brown color. Before the nature and properties of tannins were determined, the tannins in *Areca catechu L.* seeds were used for dyeing clothes, plywood fabrication, and also for tanning leather.

This research studies the effect of particle size on the efficiency of Accelerated Solvent Extraction (ASE) of catechin from dried *Areca catechu L.* seeds. Particle size reduction of plant seeds has become a vital aspect that has to be considered and also has a significant effect in the extraction of catechin compound. Generally, the efficiency of extraction processes is mainly affected by some extraction parameters such as temperature, time, types of solvent and particle size (Franco *et al.*, 2007). Moreover, the sample particle size of the plant seeds and the extraction

temperature significantly affect the efficiency of the extraction process. Particularly, the sample particle size can influence and control the mass transfer kinetics and the access of solvent (water) into the soluble components. It is also one of the important studied criteria as it can affect the extraction rate, diffusion rate and extracability.

ASE technically is a green technology, was applied in order to extract the dye pigment. The extraction process used water as a solvent since water is an environmentally friendly solvent compared to other chemicals such as methanol, ethanol, ether and et cetera. ASE is one of the most up-to-date solid-liquid extraction techniques (Li *et al.*, 2003). The use of ASE is good in operation compared to traditional methods because it is able to be improved more effectively by varying some parameters, for instance, temperature, solvent, time and extraction cycles. On the other hand, the use of ASE can reduce cost and time-consumption, in which even a small amount of solvent can operate at higher of temperature and pressure as well (Oukebdane *et al*., 2010; Zaibunnisa *et al*., 2009). ASE is one of the famous methods of extraction which consume limited amount of solvent in recovering semi volatile compounds (Fisher *et al.*, 1997; Dean, 1996; Saim *et al.*, 1998).

In addition, the process of using ASE is basically operated at high pressure and temperature, normally above the boiling point of the solvent (Cho *et al., 2007)*. In fact, the attraction ability of solvent to attract the solute will increase when the extraction process is operated at high temperature. It may also lead to the decrease of the viscosity of solvents. Therefore, penetrations of the solvent into the pores will become higher and better, so as to encourage wetting of the matrix particles in order to attain high recovery (Richter *et al.*, 1996). The applications of high pressure smoothen the progress of the extraction of solute from the samples, due to the improvement of the accessibility of solvent to reach the solute trapped in the matrix pores.

In this research, ASE was applied in the extraction of catechin at an extraction temperature of 140^{o}C, 10 min extraction time, pressure of 1500 psi, 60% flush volume and one static cycle. The amount of catechin present in the extracted was quantified via Liquid Chromatography-Time of Flight-Mass Spectrometry (LC-TOF-MS).

■2.0 MATERIALS AND METHODS

2.1 Materials

Dried *Areca catechu L.* seeds were purchased in a large quantity of about 5 kg from Pulau Pinang. Methanol and acetonitrile of HPLC grade (Merck, Darmstadt) were used for the analysis of Catechin compound. Standard (+)-Catechin hydrtae was purchased by Sigma Aldrich.

2.2 Methods

2.2.1 Particle Size Reduction

In this research, mature and dried *Areca catechu L.* seeds were used. They were obtained in large quantity, about 5 kg in order to ensure the samples used were from the same source. Then, the seeds were ground into powder. Particle size distribution was determined using Vibrator Sieve Shaker. The seeds were separated into four different sizes which were 500 µm, 250 µm, 125 µm and 75 µm in order to determine the best particle size. In order to maintain the freshness, the samples were kept in the freezer at temperature of -20^{o}C. According to Leland *et al*., (2006) the ideal temperature to maintain the freshness was -20^{o}C.

2.2.2 Accelerated Solvent Extraction

The samples (4 grams) of *Areca catechu L.* powder were mixed with diatomaceous earth (DE) with 1:1 ratio and were filled into 34 mL extraction cell size. A heating process was carried out for about 7 min and the extraction time was carried out for 10 min with 60% of flush volume of the solvent. Nitrogen was purged for 100 seconds. The extraction was conducted at the temperature, time, pressure, static time and cycle of 140^{o}C, 1500 psi, 10 minutes and 1 cycle respectively.

2.2.3 Analysis

Calibration Curve of Catechin

A 200 ppm stock solution of Catechin standard was prepared in methanol of analytical grade. Standard solutions were prepared by diluting the stock solution with methanol in four different concentrations of 100 ppm, 50 ppm, 25 ppm, 10 ppm and 1 ppm. The calibration curve was attained by plotting the concentration of Catechin standard (µg/mL) to the peak area of standard Catechin and the linear regressions and correlation coefficients were calculated.

LC -TOF-MS

Liquid Chromatography-Time of Flight-Mass Spectrometry (LC-TOF-MS) analysis was performed using an Ultimate 3000 (Dionex) with a micrOTOF-Q II (Bruker) instrument. An Acclaim Polar Advantage II of C_{18} (2.1 × 150 mm) was used with a flow rate of 0.2 ml/min. A 2µl solution was injected into the column, and separation was carried out at 35◦C using gradient elution where 0.1% Formic Acid in water (A) and pure Acetonitrile (B) were used as mobile phases. The gradient program was started from 10% to 90% B in 15 min, holding on with 90% of B for 5 min and after 10 min, went back to 10% of B for a total LC-MS run time of 30 min. The system was operated in positive ion polarity and a full scan analysis was performed beginning from 50 m/z which and ended at 1500 m/z. The samples were put into a dry heater at 180^{o}C and nitrogen was used as the desolvation gas (8 L/min).

■3.0 RESULTS AND DISCUSSIONS

3.1 Effect of Particle Size on the Percentage of Overall Extracted Yield

Particle size had a significant effect in the extraction of catechin from *Areca catechu* seeds. The values of catechin extract, ranging from particle size 75 µm until 500 µm are presented in Table 1 and Figure 1. It was found that the highest oil yield extracted was 1.20 g (30.00% oil yield) at 125µm of particle size. Meanwhile, the lowest oil yield found was 0.22 g (5.50% oil yield) with very fine particle size of 75 µm. The yield obtained from the extraction was lower compared to the other range of particle size, with a higher surface area to volume ratio and it was different from the theory which states that increasing the surface area produce high extracted yield.

In this study, it was observed that when the particle size was too fine, it would cause the sample in the extraction cell to be compact and form hard lump when in contact with the solvent at high pressure and temperature. On the other hand, it showed a significant reduction in the sample bed permeability, since it obstructed the interaction of the particles with the solvent molecules. Mukhopadhyay (2000) stated that fine particles would

reduce the transmission of charge in the solute mass transfer rate. Therefore, the solvent ability became weaker and lower in order to attract the solute.

Table 1 Catechin extracted value from various particle sizes

Particle Size (µm)	Oil Yield (g)	Global Oil Yield (%)	Catechin Yield (mg of catechin per g of sample)
75	0.22	5.50	0.0153
125	1.20	30.00	0.0375
250	1.09	27.25	0.0433
500	0.99	24.75	0.0515

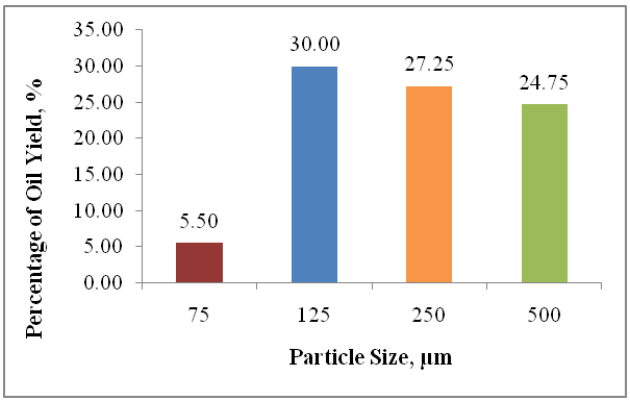


Figure 1 Percentage oil yield of various particle sizes

The results from the range of particle size of 500 µm to 125 µm complied with the theory. The smaller particle size gave more yield because of the higher surface area to volume ratio that in turn enhanced the contact between the solvent molecules and plant material during the extraction process. When the size of solute particle increases, the path between the inside of the particle and surface particle also increases, consequently lowering the extraction of the solute. Furthermore, when the size of the particle is small, it would result in relatively large surface area, hence increasing the contact area in between the solvent (Park *et al*., 2007). Size reduction also helps for easier accessibility of the soluble substrates that are otherwise located deep inside the plant matrix (Takeuchi *et al*., 2009). Moreover, the diffusion path for the compound inside the particle was reduced and thereby facilitating the extraction to be easier and faster (Jadeja *et al*., 2011).

3.2 Effect of Particle Size on the Catechin Yield in *Areca Catechu*

The LC-TOF-MS method was used for the quantitative determination of (+)-Catechin. The working concentration range was between 1–200 µg/mL (ppm). Within this interval, the calibration curve were linear with correlation coefficient (R^2) = 0.9932. The catechin content was expressed in mg of Catechin/g of sample.

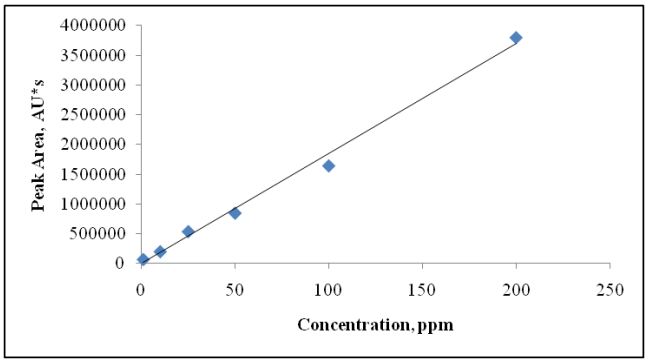


Figure 2 Calibration curve for catechin

The identification and quantification of catechin was analyzed by using LC-TOF-MS analysis. Figure 3 to 6 illustrate the retention time of the catechin standard and the catechin extract appeared at 9.1 min and 9.2 min respectively. The catechin compound was identified when their retention time was under the tolerance of standard retention time.

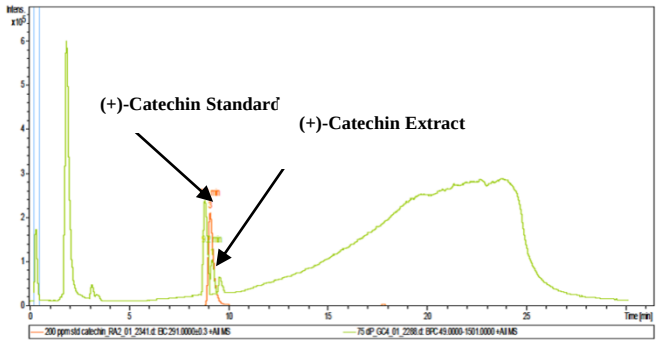


Figure 3 LC-TOF peak chromatograms of catechin standard and catechin extract (75 µm)

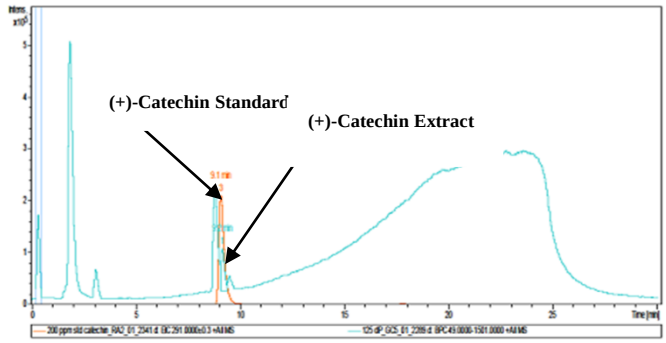


Figure 4 LC-TOF peak chromatograms of catechin standard and catechin extract (125 µm)

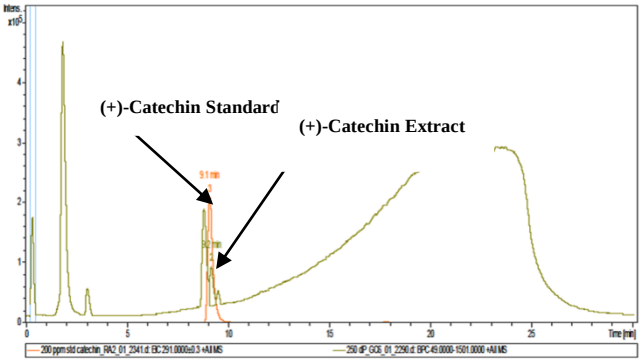


Figure 5 LC-TOF peak chromatograms of catechin standard and catechin extract (250 µm)

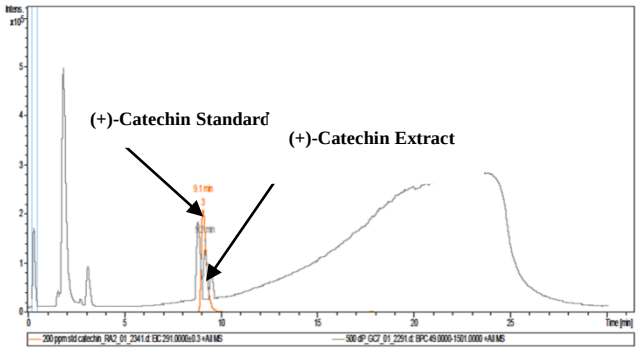


Figure 6 LC-TOF peak chromatograms of catechin standard and catechin extract (500 µm)

The result presented in Figure 7 shows that the particle size had a significant influence on the yield of the catechin compound. The highest catechin content was quantified at 500 µm which was 0.0515 mg of catechin/g of the sample. As the particle size decreased from 500 µm to 75 µm, the amount of catechin yield also decreased. According to Hu *et al.* (2012) the content of catechin will decrease when the particle size is reduced. The extraction of catechin was interrupted when it came to the use of fine particles size which led to a low yield of catechin compound. These fine particles settled into the bottom and formed a sediment at the bottom of the extraction cell, which reduced the flow-through of water and subsequently the sample did not efficiently interact with the water (Vuong *et al.*, 2011). The extraction process of ASE was operated at high pressure and temperature, where these fine particles would form hard lump and lower the ability of the solvent to extract molecules.

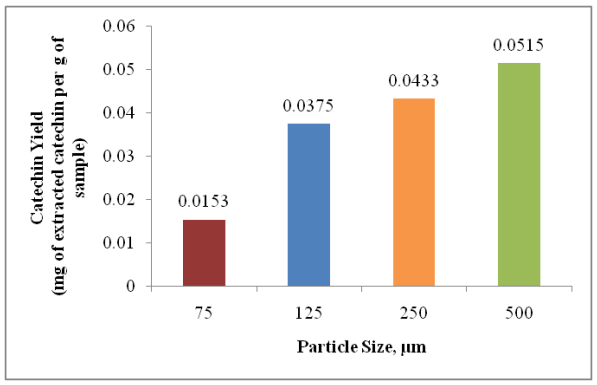


Figure 7 Catechin yield of various particle size

The use of finest sample particle also had caused some problems during the extraction where the pump failed to function properly. With the occurrence of this problem, it was observed that the extracted solution were reduced into half than the use of larger particle size (>75 µm) as shown in Figure 8. By physical observation (Figure 8), there was a clear difference on the color of the extracted solution for different particle size fractions. The color of the extracted changed from brown to dark brown starting with particle size 75 µm to 500 µm. This might be due to the concentration of catechin which tended to be higher as the particle size increased from 75 µm to 500 µm with the highest catechin yield was found to be 0.0515 mg of extracted catechin/g of sample. Hence, the best of particle size of 500 µm has been used for the extraction of catechin from *Areca catechu L.* seeds by using ASE.

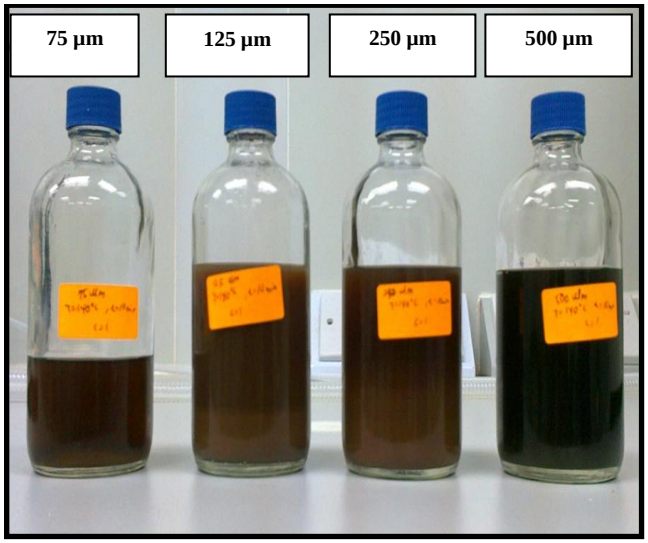


Figure 8 Color of extracted yield for four different particle size

4.0 CONCLUSION

Catechin has been successfully extracted from *Areca catechu L.* seeds with different particle sizes which are 500 µm, 250 µm, 125 µm and 75 µm. The highest yield is obtained from *Areca catechu L.* seeds having small particle size (125 µm) with 30.00% overall oil yield. In general, when the particle size reduced, the extraction efficiency will increase. However, if the particle size is too fine, it would lower the extraction efficiency. Catechin is identified as the target compound present in the extracted *Areca catechu L.* seeds by using LC-TOF-MS analysis. The highest catechin yield obtained has a large particle size (500 µm) with 0.0515 mg of catechin/g of sample. Therefore, the best of particle size has been identified at 500 µm for the extraction of catechin by using Accelerated Solvent Extraction.

Acknowledgement

The authors gratefully acknowledge the Centre for Lipids Engineering and Applied Research (CLEAR) Universiti Teknologi Malaysia for the financial support (Q.J130000.7125.00H28), and the Centre for Herbal and Standardization (CHEST) Universiti Sains Malaysia for making this study possible.

References

[1] Azeez, S., Amudhan, S., Adiga, S., Rao, N., Rao, N. and Udupa, L. A. 2007. Wound Healing Profile of Areca Catechu Extracts on Different Wound Models in Wistar Rat. *Medical Journal*. 39 (1): 48–52.

[2] Cho, S. K., Abd El-Aty, A. M., Choi, J. H., Kim, M. R. and Shim, J. H. 2007. Optimized Conditions for The Extraction of Secondary Volatile Metabolites in Angelica Roots by Accelerated Solvent Extraction. *J. Pharm. Biomed. Anal*. 44: 1154–1158.

[3] Dean, J. R. 1996. Accelerated Solvent Extraction of Polycyclic Aromatic Hydrocarbons from Contaminated Soil. *Anal. Commun*. 33: 191–192.

[4] Fisher, J. A., Scarlett, M. J. and Stott, A. D. 1997. Accelerated Solvent Extraction: An Evaluation for Screening of Soils for Selected U.S. EPA Semivolatile Organic Priority Pollutants. *Environmental Sci. Technol.* 31 (4): 1120–1127.

[5] Franco, D., Pinelo, M., Sineiro, J. and Nunez, M. J. 2007. Processing of Rosa Rubiginosa: Extraction of Oil and Antioxidant Substances. *Bioresource Technol*. 98: 3506–3512.

[6] Ho, C. T., Lee, C. Y. and Huang, M. T. 1992. Phenolic Compounds in Food and Their Effects on Health II Antioxidants and Cancer Prevention. American Chemical Society, Washington. *Free Radical Biology and Medicine*. 16(5): 669–670.

[7] Hu, J., Chen, Y. and Ni, D. 2012. Effect of Superfine Grinding On Quality and Antioxidant Property of Fine Green Tea Powders. *Food Sci. Technol*. 45: 8–12.

[8] Jadeja, G. C., Maheshwari, R. C. and Naik, S. N. 2011. Extraction of Natural Insecticide Azadirachtin From Neem (Azadirachta Indica A. Juss) Seed Kernels Using Pressurized Hot Solvent. *J. Supercritical Fluid*. 56: 253–258.

[9] Lee, K. K. and Choi, J. D. 1999. The Effects of Areca Catechu L. Extract on Anti-Aging. *International J. Cosmetic Sci*. 21: 285–295.

[10] Leland, J. C., Kirakosyan, A., Kaufman, P. B., Warber, S. L., Duke, J. A. and Brielmann, H. L. 2006. *Natural Products from Plants*. 2nd ed. Boca Raton: CRC Press Taylor & Francis Group.

[11] Li, Ken, Landriault, M., Fingas, M. and Llompart, M. 2003. Accelerated Solvent Extraction (ASE) of Environmental Organic Compounds in Soils Using A Modified Supercritical Fluid Extractor. *J. Hazardous Materials*. 102: 93–104.

[12] Mukhopadhyay, M. 2000. *Natural Extracts using Supercritical Carbon Dioxide*. CRC Press, Boca Raton.

[13] Oukebdane, K., Portet-Koltalo, F., Machour, N., Dionnet, F. and Desbene, P. L. 2010. Comparison of Hot Soxhlet and Accelerated Solvent Extractions with Microwave and Supercritical Fluid Extractions for The Determination of Polycyclic Aromatic Hydrocarbons and Nitrated Derivatives Strongly Adsorbed on Soot Collected Inside a Diesel Particulate Filter. *Talanta*. 82: 227–236.

[14] Park, H. S., Choi, H. K., Lee, S. J., Park, K. W., Choi, S. G. and Kim, K. H. 2007. Effect of Mass Transfer on the Removal of Caffeine from Green Tea by Supercritical Carbon Dioxide. *J. Supercritical Fluids.* 42: 205–211.

[15] Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N. and Pohl, C. 1996. Accelerated Solvent Extraction: A Technique for Sample Preparation. *Anal. Chem.* 68(6): 1033–1039.

[16] Saim, N., Dean, J. R., Abdullah, M. P. and Zakaria, Z. 1998. An Experimental Design Approach for the Determination of Polycyclic Aromatic Hydrocarbons from Highly Contaminated Soil Using Accelerated Solvent Extraction. *Anal. Chem.* 70(2): 420–424.

[17] Takeuchi, T. M., Pereira, C. G., Braga, M. E. M., Marostica, M. R., Leal, P. F. and Meireles, M. A. A. 2009. *Extracting Bioactive Compounds for Food Products-Theory and Applications*. Boca Raton: CRC Press.

[18] Vuong, Q. V., Golding, J. B., Stathopoulos, C. E., Nguyen, M. H. and Roach, P. D. 2011. Optimizing Conditions for The Extraction of Catechins from Green Tea Using Hot Water. *J. Sep. Sci.* 34: 1–8.

[19] Zaibunnisa, A. H., Norashikin, S., Mamot, S., Osman, H. 2009. An Experimental Design Approach for The Extraction of Volatile Compounds from Turmeric Leaves (Curcuma domestica) Using Pressurised Liquid Extraction (PLE). *Food Sci. Technol*. 42: 233–238.